Extraction of betulin from bark of *Betula platyphylla* by supercritical carbon dioxide extraction

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Abstract: Betulin, which is a medicinal pentacyclic triterpene, is abundant in the bark of white birch (*Betula platyphlly*). The bark of birch was collected at Tayuan Forest Farm of Jiagedaqi, Heilongjiang Province in September 2000. Supercritical fluid extraction (SFE) that is a new separation technology has been used for the processing pharmaceutical and natural products. In this paper, the extraction of betulin from the bark of birch by supercritical CO_2 extraction was studied. The authors investigated and analyzed a few parameters such as modifier dosage, extraction pressure and extraction temperature. The optimal extraction conditions showed that the modifier dosage used for per gram bark powder was 1.5 mL, the extraction pressure was at 20 Mpa, and the extraction temperature was at 55 °C. The velocity of flow of liquid CO_2 was at 10 kg/h. The pressure and temperature in separation vessel were at 5.5 Mpa and 50 °C, respectively.

Keywords: *Betula platyphlly*; Betulin; Supercritical CO_2 extraction
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Introduction

Betulin (lup-20 (29)-en-3β, 28-diol), as a pentacyclic triterpene with the lupane skeleton, was also named betulenol, trochton, birkenkampher, and (coryli-) resinol (Hayek *et al.* 1989), and its content is abundant in the bark of white birch (*Betula platyphlly*), (Fig. 1). Betulinic acid, a derivative of betulin, has been found that it selectively kills human melanoma cells and the healthy cells were kept alive. So Betulinic acid's specific effect is unique, compared with many chemotherapy drugs, including camptothecin, ellipticine, mithramycin A, etoposide, vinblastine, and vincristine (Pisha *et al.* 1995). Betulinic acid has also been found that it can retard the progression of HIV 1 infection, which eventually leads to AIDS, by preventing the formation of syncytia (cellular aggregates), (Mayaux *et al.* 1994; Fujioka *et al.* 1994).

The conventional extraction methods, such as multi-step, liquid-liquid extraction and Soxhlet extraction, were often lack of precision, lasted in long time and were in high expenditure. These methods were required for treating raw materials in high temperatures and in bright light, which often makes the heat-sensitive and photosensitive compounds decompose. Supercritical fluid extraction (SFE), a new separation technology, has been used in the processing pharmaceutical and natural products. CO_2 is a promising solvent since it is inexpensive, nontoxic, inflammable, and has a low critical temperature and a moderate critical pressure (Tsuda *et al.* 1995). In this paper, the extraction of betulin from bark of birch (*Betula platyphylla* Suk.) by supercritical CO_2 extraction (SC-CO_2 extraction) was studied, and the optimal condition for the extraction of betulin was explored.

The bark of birch is the waste during the manufacture of birch wood, but it is valuable for extracting betulin from the bark in industry. Betulin, as the pre-compound of betulinic acid, becomes more important in the pharmaceutical utilization.

Materials and methods

Plant material

Birch bark collected at Tayuan Forest Farm of Jiagedaqi, Heilongjiang Province in September 2000 was dried at 70 °C to be constant weight. Dried samples were ground and the particles were 60 meshes.

Reagent

CO_2 purity was for 99.5% from Harbin Gas Company, and the ethanol purity was for 95% from Harbin Chemical Factory.

Extraction apparatus

SC-CO_2 Extraction was performed by using HA121-50-01 supercritical extraction equipment (Haian Petroleum Scientific Research Equipment Factory) with 1-L extraction vessels of stainless steel.

SC-CO_2 Extraction

After birch bark of 150 g was packed in the extraction vessel, SC-CO_2 extraction was preformed. Liquid CO_2 was compressed and then pumped through the extraction vessel. The velocity of flow of liquid CO_2 was at 10 kg/h. The

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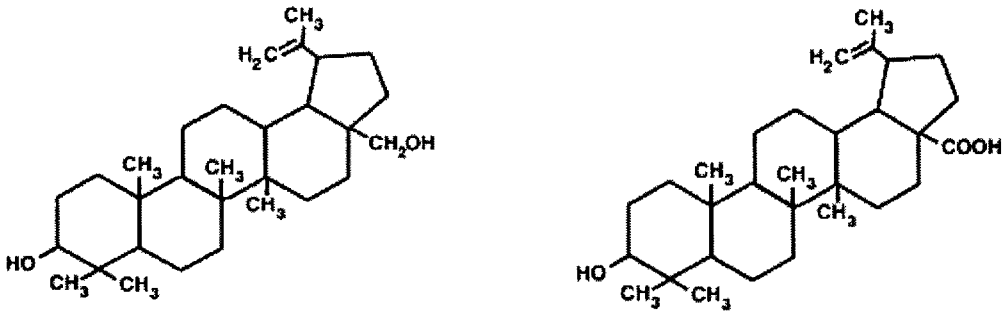
pressure and temperature in separation vessel were at 5.5 Mpa and 50 °C, respectively.

Extraction modifier

Ethanol was used as the extraction modifier.

Determination

Betulin content in the SC-CO_2 extracts was tested by GC-MC (Platform II GC-LC-MS instrument, VG Int., UK) based on the O'-Connell *et al.* method (1988).



Betulin Betulinic acid

Fig. 1 Chemical structures of Betulin and Betulinic acid

Results and discussion

Modifier amount

Controlled extraction pressure was at 25 Mpa and the temperature was at 50 °C. The five different modifier amounts were selected (Fig. 2). The results showed that the bigger modifier amount was added, the better extraction yield was gotten. However when the modifier dosage was used from 1.5 mL to 2 mL per gram bark powder, the yields of betulin had a little difference. On the other hand, the more modifier was added, the longer extraction time was needed. The more energy was consumed and the more solvent consumption was occurred. Considering all these aspects, the modifier dosage was 1.5 mL per gram bark powder, which was the optimal proportion, when the extraction temperature was at 55 °C.

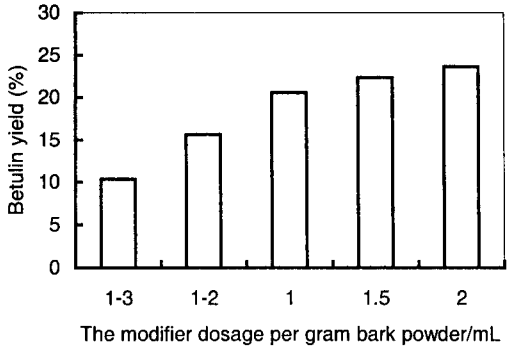


Fig. 2 The influence on the modifier amount

Influence of extraction pressure on extraction yields

The effects of five different extraction pressures on extract yields were investigated (Fig. 3). The results showed that betulin extraction yields were stable when the extraction pressure was over 20 Mpa. Although higher extraction pressure could speed the extraction, the higher equipment pressure-burdened capability was demanded. So the optimal extraction pressure should be at 20 Mpa.

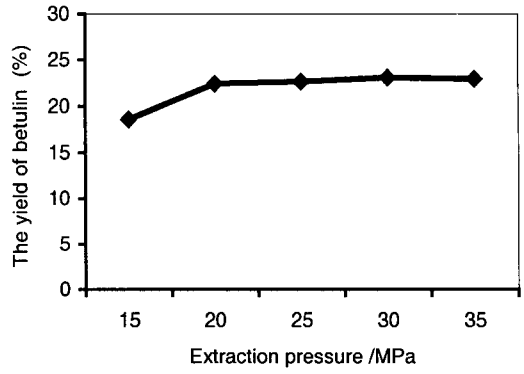


Fig. 3 The influence of the extraction pressure

Influence of extraction temperature on extraction yield

The extraction temperature is another important factor for SC-CO_2 extraction. The results showed that the extraction yield firstly increased with the increase of extraction temperature, and than decreased with the further increment of extraction temperature (Fig. 4). The optimal condition was at 55°C as the extraction temperature.

Conclusions

From our experimental results, we can see that betulin can be extracted from the bark of white birch (*Betula platyphylla*) by SC-CO_2 extraction. The optimal conditions showed that the modifier dosage was 1.5 mL per gram bark powder, the extractive pressure was at 20 Mpa, and

the extractive temperature was at 55 °C.

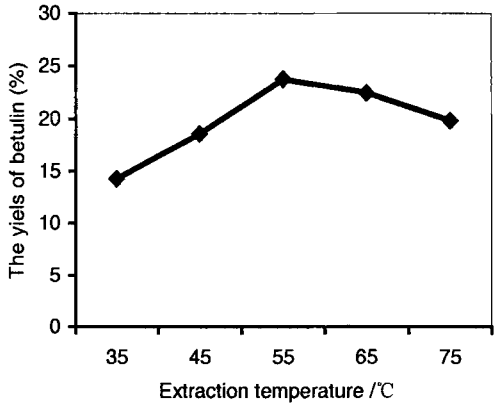


Fig. 4 The influence of the extraction temperature

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